United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health and Science
Laboratory QA/QC Division
950 College Station Road
Athens, GA 30605

Microbiology Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 4A.01

Title: FSIS Procedure For the Use of *Salmonella* Rapid Screening Immunoassay Kits

Effective Date: 10/25/02

Description and purpose of change(s):

The Microbiology Laboratory Guidebook (MLG) chapters are currently under revision. The formatting is being changed to meet the requirements of the laboratory's document control system. Additional content is being added to meet the requirements of ISO 17025. MLG 4A has been revised to include a statement of the method detection limits, a section on safety precautions and a revised section on quality control practices. It has also been expanded to include the use of rapid immunoassay kits for analysis of processed egg products.

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Title: FSIS Procedure For the Use of *Salmonella* Rapid Screening Immunoassay Kits		
Revision: 01	Replaces: Original	Effective: 10/25/02

Procedure Outline

4A.1 Introduction

4A.1.1 General

This method describes the use of commercial, rapid screening immunoassays to screen-test processed egg products, raw foods, and raw swab, sponge, and rinse samples for *Salmonella*. All kits must have passed one of the three AOAC methods validation programs (Performance Tested Methods, Official Methods of Analysis, or Peer-Verified Methods), or equivalent program with adequate supporting data, and must meet the criteria for sensitivity and specificity as specified in the MLG 4 Section 4.4.5. All samples identified as presumptively positive for *Salmonella* by these tests are subject to cultural confirmation.

4A.1.2 Limits of Detection

This method, as evaluated with a specific lot of *Salmonella* Assurance® EIA from BioControl Systems, Inc. (Bellevue, WA), was found to have a sensitivity of at least 97% for samples containing less than 3 colony forming units (cfu)/g in a 25 gram sample.

Salmonella counts represent the inoculum before freezing. Samples were tested after being frozen. Specificity was > 90%. Sensitivity and specificity may vary from lot to lot and for kits from different manufacturers. Sensitivity and specificity must be determined for each lot before use in this assay. All lots of kits used must meet MLG 4 Section 4.4.5 guidelines for acceptability (sensitivity of ≥ 97%, specificity ≥ 90%, false-negative rate ≤ 3%, and false-positive rate ≤ 10%.)

4A.2 Safety Precautions

Salmonella are generally categorized as BioSafety Level 2 pathogens. CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Salmonella* are used. All available Material Safety Data Sheets (MSDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material should read all MSDS sheets.

4A.3 Quality Control Procedures

4A.3.1 Culture Controls

Include at least three method controls in all analyses. These controls must include a *Salmonella* spp. H_2S-negative culture, a *Salmonella* spp. H_2S-positive culture and an uninoculated media control. To facilitate identification of control isolates, the laboratory may use strains of uncommonly found serogroups. S. Abaetetuba, serogroup F, is suggested as a readily available, H_2S-positive, culture that is not commonly found in meats or meat products. *Salmonella* serotype Choleraesuis is typically negative for H_2S production. These cultures may be obtained from ATCC. Other serotypes may be found that have aberrant H_2S-negative strains. The inoculum level for the positive controls should approximate 30 to 1000 cfu per sample. A 1 microliter loop-full of a suspension of a fresh culture equivalent to a 0.5 McFarland Standard may be used for this purpose. Alternatively, commercially prepared bacterial pellets containing concentrations of 100-1000 cfu/pellet may be used according to the manufacturer's instructions. The control cultures should be inoculated into either a meat matrix or the matrix that is being analyzed. Incubate the controls along with the samples, and analyze them in the same manner as the samples along with the samples through all procedures. Include at least one complete set of 3 controls in each 96-well microtube plate or run. Confirm at least one isolate from each positive control sample. In the absence of a positive test sample, control cultures may be terminated at the same point as the sample analyses.

4A.3.2 Kit controls

In addition to the culture controls, include the appropriate kit controls as described by the manufacturer, or on every microtiter plate at a minimum.

4A.4 Equipment, Reagents, and Media

In addition to equipment, reagents, and media used in analysis of samples as described in MLG 4, the following materials may be needed.

a. DIAS™, Dynex Automated ImmunoAssay System (optional)
b. Autoclave, boiling water bath, or equivalent device capable of heating to 100°C.
c. Water bath, 42 ± 0.5°C
d. Microtubes, 1 to 2 ml, for 96 well format
e. Microtube 96 well format boxes/racks
f. Micropipettor, capable of accurately dispensing 100 µl, 50 µl, and 250 µl, as needed
g. Micropipet tips
h. M Broth

4A.5 Culture Methods

Perform sample inoculation and pre-enrichment in buffered peptone water (BPW) as described in MLG 4 Section 4.5 for the type of product to be analyzed. After incubation of the pre-enrichment culture in BPW, continue as described below.

4A.5.1. Raw foods

a. Selective enrichment

Transfer 0.5 ± 0.05 ml of the BPW pre-enrichment culture into 10 ml of TT broth (Hajna). Transfer 0.1 ± 0.02 ml of the BPW pre-enrichment culture into 10 ml of modified Rappaport Vassiliadis (mRV) broth (R10 or RVS). Incubate the TT and mRV enrichment cultures in an incubator at 42 ± 0.5°C for 22-24 h, or in a water bath at 42 ± 0.5°C for 18-24 h.

b. Post enrichment

Transfer 0.5 ± 0.05 ml from the TT broth into 10 ml of M Broth. Transfer 0.5 ± 0.05 ml from the mRV broth into the same M Broth tube. Retain the original selective enrichment broths at 2-8°C for possible confirmation of presumptive positive samples.

c. Incubate the M Broth enrichments in a water bath at 42 ± 0.5°C for 5-8 h.

4A.5.2 Processed Egg Products

a. Selective enrichment

Transfer 0.5 ± 0.05 ml of the BPW pre-enrichment culture into 10 ml of TT broth (Hajna). Transfer 0.1 ± 0.02 ml of the BPW pre-enrichment culture into 10 ml of modified Rappaport Vassiliadis (mRV) broth (either R10 or RVS). Incubate the TT and RV enrichment cultures in an incubator or water bath at 42 ± 0.5 C for 5 to 8 h. If an air incubator is used, the TT and RV must be pre-warmed to 42°C before they are inoculated.

b. Post enrichment

Transfer 0.5 ± 0.05 ml from the TT broth into 10 ml of M Broth. Transfer 0.5 ± 0.05 ml from the RV broth into the same M Broth tube. Retain the original selective enrichment broths at 2-8°C for possible confirmation of presumptive positive samples.

c. Incubate the M Broth enrichments at 35-37°C for 14 to 18 h.

4A.6 Preparation of Samples for ELISA: Raw and Processed Products

4A.6.1 Sample Portioning

Following M Broth incubation, vortex or mix the tubes and transfer 0.75 to 1.0 ml of each sample and control M Broth into labeled tubes or, if using the DIAS™, into the appropriate wells of a 96 well microtube block as diagrammed in the DIAS™ template.

Retain the original M Broth enrichments at 2-8°C for confirmation of presumptive positive tests.

4A.6.2 Heat Inactivation

Heat the M Broth aliquots at approximately 100°C for 20 minutes. This may be accomplished using an autoclave on isotherm (flowing steam) cycle, a boiling water bath, or heating block set to 100°C. Cool the tubes to 15 - 37°C before performing the ELISA. Heat-treated samples may be refrigerated at 2-8°C for up to 4 days prior to testing.

4A.7 ELISA Rapid Screening Test Procedure

Follow the manufacturer's directions for preparing reagents, performing the ELISA, reading the plates and interpreting the results. If the DIAS™ is used, the equipment must be set up, and operated, and records must be documented according to the manufacturer's requirements and laboratory work instructions. Include all blanks, kit positive controls and kit negative controls according to kit instructions.

4A.8 Cultural Confirmation of Screen Test Positive Samples

Cultural isolation and identification must be performed to confirm presumptive positive results from the screen test. Test the non-heated, reserved M broth and/or selective enrichment cultures as follows.

a. Vortex or mix each presumptive-positive, retained, non-heated, enriched M broth and/or TT and RV broth. Using an inoculating loop, streak each onto both BGS and DMLIA, or onto BGS and XLT4 agar plates. Do not subdivide plates for streaking multiple samples; streak the entire plate for isolation with a single sample broth.

b. Incubate the plates at 35 ± 1°C.

c. Examine the plates after 18-24 h of incubation for typical *Salmonella* colonies. Re-incubate all plates and re-examine them the following day.

d. Select and confirm suspect colonies as described in MLG 4 Section 4.6 et seq. for isolation and confirmation.

4A.9 Selected References

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 1999. BioSafety in Microbiological and Biomedical Laboratories, 4th ed. U.S. Government Printing Office, Washington, D.C. (or at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

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Sperber, W. H., and R. H. Deibel. 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. **17**:533-539.